CHANGES IN THE DISTRIBUTION OF NITROGEN DURING GROWTH AND METAMORPHOSIS OF THE HOUSEFLY, MUSCA DOMESTICA (LINNÆUS)*

By Robert J. DelVecchio
Department of Biology, Fordham University

Needham (1929), pointed out that when histolysis of larval structures occurs, their constituent proteins are presumably broken down resulting in a raised proportion of proteoses, peptones, and amino acids. As imaginal organs are constructed, this trend should be reversed, and the relative concentration of complete protein should increase. In addition, if some nitrogenous compounds are destroyed during metamorphosis, the fact that nothing, except gases, may leave the body during the pupal stage, should result in an increase in the concentration of end-products and in the proportion of non-protein nitrogen from this source.

Anderson (1948) working on the changes in the distribution of nitrogen in the Japanese beetle, *Popillia japonica,* showed that there was no significant loss of nitrogen during the course of metamorphosis. However, at pupation there was a sudden large decrease in the nitrogen of the water-insoluble fraction, followed by gradual increases during subsequent days of pupal life. An increase in amino acid nitrogen occurred at pupation followed by a decrease, which became more gradual during the remainder of pupal life. Soluble protein, proteose and peptone nitrogen decreased significantly between larval and early prepupal stages. They showed no change in the late prepupa but subsequently rose to a maximum percentage of the total nitrogen on the second to third day of the pupal period at 25° C. By the sixth to seventh day they had returned to the former level and maintained this concentration through emergence. These complimentary shifts may be interpreted as indicative of the destruction of mature larval tissues at pupation, followed by gradual

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construction, from the materials thus made available, of the definitive structures of the adult.

Most investigators confined their analyses to larvæ, pupæ or adults, without making a complete analytical study during these stages to determine exactly when the changes first occurred and when they are reversed. Therefore it was decided to investigate the changes in nitrogenous components in the housefly, *Musca domestica,* and to analyze insects during each day of the larval and pupal periods, and finally as young and old adults. These data should be of value in comparative studies of the developmental physiology of insects in general.

MATERIAL AND METHODS

Larvæ of the housefly were raised on whole milk. A finger-bowl containing cotton saturated with this food served as the feeding medium. Eggs were transferred daily to a humidifier regulated at approximately 30° C. and a humidity near saturation. The eggs hatched in approximately 24 hours under these conditions, and the time of hatching was recorded. In this manner carefully timed records, within 24 hours, were obtained for each group of experimental animals. The flies were tested in samples of approximately 100 mg. at the following stages: one-, two-, three-, four-, five-, and six-day larvæ; one-, two-, three-, and four-day pupæ; newly emerged adults, and old adults (seven to ten days after emergence).

Fractionation was accomplished by the techniques used by Ludwig and Rothstein (1952), Anderson (1948), and also by a modification of these techniques. By the first method, Fraction A (lipid nitrogen) was extracted with a solution of ether-alcohol. Fraction B was then extracted with hot water and separated from Fraction C by the addition of sodium tungstate and sulfuric acid. Fraction B probably contained amino acids, ammonia, urates, and urea, while Fraction C (water-soluble nitrogen precipitated by tungstic acid) probably consisted of soluble proteins, proteoses, peptones and polypeptides. Fraction D, the residue remaining after the previous extractions, contained complex proteins and chitin. With the technique used by Anderson (1948), cold water extraction was made but no lipid nitrogen was removed. Hence, this method gave Fractions B, C, and D. The

third method consisted of cold water extraction prior to lipid removal with alcohol-ether. The remaining fractionations were

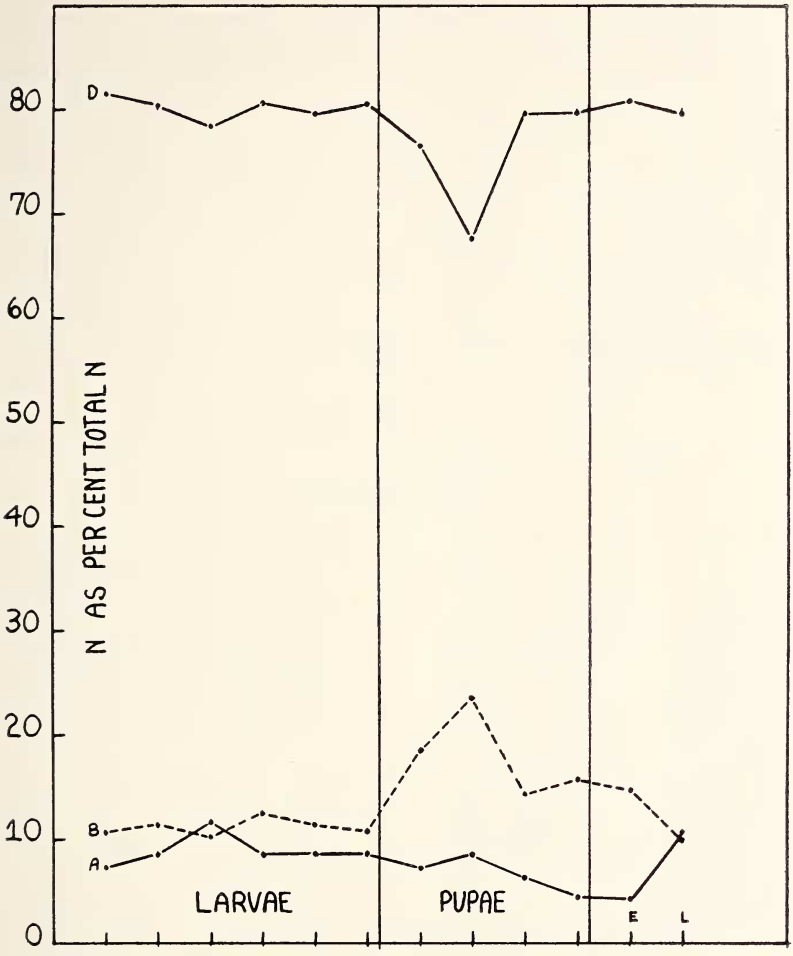

Fig. 1. Graph showing the per cent of total nitrogen of the various fractions at different stages of metamorphosis (technique used by Ludwig and Rothstein 1952).

performed according to the procedure of Ludwig and Rothstein (1952). This method yielded Fractions A, B, C, and D. The Kjeldahl procedure was employed to make the nitrogen determi-

nations. A minimum of 10 determinations was made on each day of growth and metamorphosis tested.

OBSERVATIONS

No loss of nitrogen occurred during the process of metamorphosis from the larva to pupa. However, there was an increase in the total nitrogen content per 100 mg. The average nitrogen of all three series of experiments increased from 1.35 per cent in the six-day larva to 2.27 in the one-day pupa, and 2.55 per cent in the young adult. This increase in total nitrogen is thought to be associated with the decrease in the amount of water, since pupae and adults contain less water than larvæ.

The changes in the distribution of nitrogen for each day of metamorphosis as derived by the technique used by Ludwig and Rothstein (1952), are shown in Table 1, and in Figure 1. Each

TABLE 1

CHANGES IN THE DISTRIBUTION OF NITROGEN DURING THE METAMORPHOSIS OF THE HOUSEFLY. NITROGEN AS PER CENT TOTAL NITROGEN AND STANDARD ERRORS.

	FRACTION A	FRACTION B	FRACTION D
1-day larva	7.22 ± .0217	10.96 ± .0107	81.81 ± .0107
2-day larva	8.78 ± .0176	11.16 ± .0220	80.04 ± .0117
3-day larva	11.30 ± .0206	10.27 ± .0135	78.44 ± .0067
4-day larva	8.79 ± .0107	12.39 ± .0220	80.71 ± .0075
5-day larva	8.89 ± .0075	11.18 ± .0082	79.92 ± .0118
6-day larva	8.92 ± .0119	10.62 ± .0119	80.45 ± .0142
1-day pupa	7.26 ± .0144	18.96 ± .0066	76.04 ± .0083
2-day pupa	8.78 ± .0120	23.92 ± .0085	67.62 ± .0119
3-day pupa	6.25 ± .0082	14.09 ± .0065	79.68 ± .0118
4-day pupa	4.30 ± .0116	16.06 ± .0118	79.51 ± .0114
Early adult	4.15 ± .0142	14.86 ± .0169	80.97 ± .0247
Late adult	10.53 ± .0219	9.67 ± .0085	79.93 ± .0221

fraction is expressed as per cent total nitrogen. Fraction A, increased during the larval period from 7.22 in the one-day, to 8.92 per cent in the six-day larvæ. During the latter part of the pupal period it decreased to a value of 4.30 per cent. No change occurred on emergence, but this fraction increased to 10.53 per cent in the old adult. Fraction B remained constant

during the larval period at approximately 11 per cent of total nitrogen. However, it increased to 23.92 per cent in the two-day pupa and then decreased to 16.06 per cent in the four-day pupa. During the adult stage, this fraction showed a further

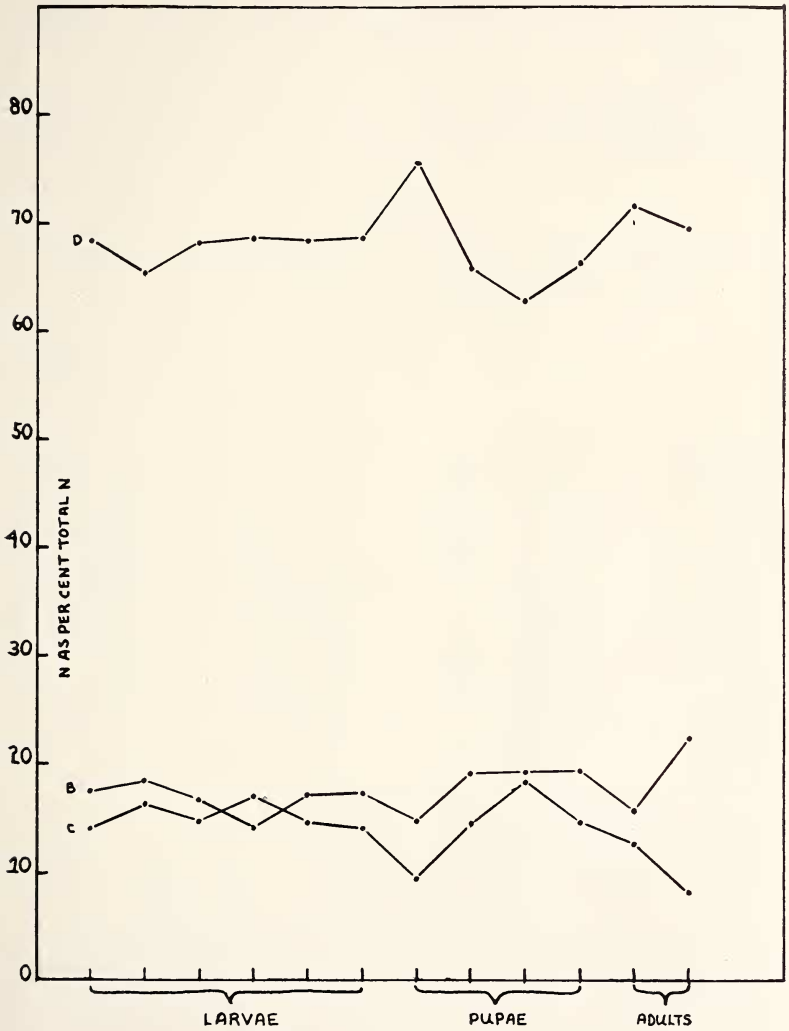

Fig. 2. Graph showing the per cent of total nitrogen of the various fractions at different stages of metamorphosis (technique used by Anderson 1948).

decrease to 9.67 per cent in the old adult. No Fraction C appeared with this procedure. Fraction D remained constant at approximately 80 per cent of the total nitrogen during the larval stage. It decreased to 67.62 per cent in the two-day pupa and returned to the larval value of approximately 80 per cent in the three- and four-day pupæ, retaining this value in the young and old adults.

The changes in the distribution of nitrogen for each day of metamorphosis as derived by the technique of Anderson (1948), are expressed in Table 2, and in Figure 2. No fraction A was

TABLE 2

CHANGES IN THE DISTRIBUTION OF NITROGEN DURING THE METAMORPHOSIS OF THE HOUSEFLY. NITROGEN AS PER CENT TOTAL NITROGEN AND STANDARD ERRORS.

	FRACTION B	FRACTION C	FRACTION D
1-day larva	17.68 ± .0141	14.01 ± .0103	68.49 ± .0107
2-day larva	18.43 ± .0179	16.37 ± .0115	65.22 ± .0115
3-day larva	16.95 ± .0117	14.89 ± .0119	68.17 ± .0141
4-day larva	14.05 ± .0115	17.17 ± .0179	68.77 ± .0177
5-day larva	17.01 ± .0063	14.77 ± .0107	68.21 ± .0177
6-day larva	17.24 ± .0107	14.14 ± .0117	68.62 ± .0083
1-day pupa	14.95 ± .0067	9.56 ± .0332	75.48 ± .0176
2-day pupa	19.08 ± .0070	14.63 ± .0035	65.92 ± .0176
3-day pupa	19.05 ± .0089	18.41 ± .0017	62.67 ± .0108
4-day pupa	19.34 ± .0067	14.59 ± .0075	66.06 ± .0108
Early adult	15.85 ± .0019	12.72 ± .0216	71.39 ± .0107
Late adult	22.38 ± .0019	8.14 ± .0107	69.48 ± .0102

obtained with this procedure. Fraction B remained relatively constant at approximately 17 per cent of total nitrogen during the larval period. Increased to 19 per cent in the two-day pupæ and retained this value until the end of the pupal stage, decreasing to 15.85 on emergence. During the adult stage it increased to 22.38 per cent. Fraction C remained relatively constant at about 14 per cent of total nitrogen during the larval stage. It increased to 18.41 per cent in the three-day pupa. This fraction then decreased steadily during the remainder of the life cycle to

14.59 in the four-day pupa, 12.72 in the young adult, and finally to 8.14 per cent in the old adult. Fraction D remained constant at 68 per cent throughout the larval period. It decreased to 62.67 per cent in the three-day pupa. This decrease was then

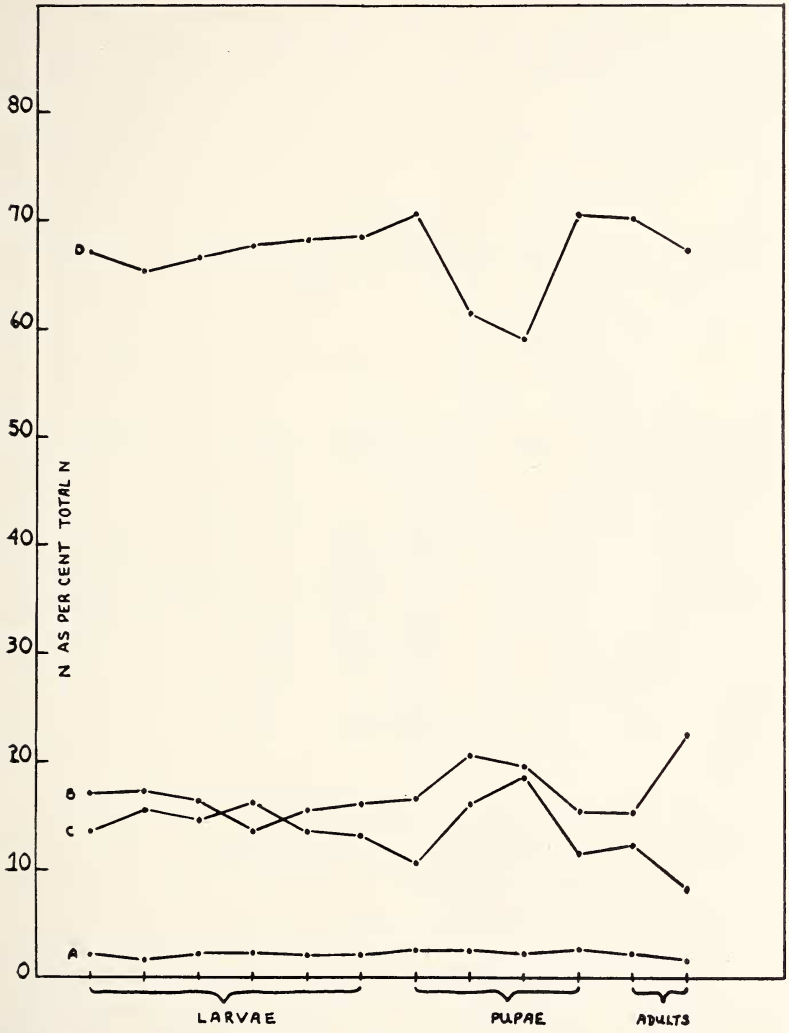

Fig. 3. Graph showing the per cent of total nitrogen of the various fractions at different stages of metamorphosis (water extraction prior to lipid extraction).

followed by an increase to 66.06 in the four-day pupa and to 71.39 per cent in the young adults.

The results obtained when the water-soluble materials were extracted prior to lipid extraction are shown in Table 3, and in Figure 3. Fraction A remained constant at approximately 2 per cent of the total nitrogen throughout the life cycle. Fraction B remained constant at 17 per cent during the first three days of the larval period, and then decreased to 13.39 per cent in the four-day larva. This decrease was followed by an increase to 16 per cent where it remained up to and including the one-day

TABLE 3

CHANGES IN THE DISTRIBUTION OF NITROGEN DURING THE METAMORPHOSIS OF THE HOUSEFLY. NITROGEN AS PER CENT TOTAL NITROGEN AND STANDARD ERRORS.

	FRACTION A	FRACTION B	FRACTION C	FRACTION D
1-day larva	2.076 ± .0117	17.19 ± .0141	13.63 ± .0103	67.09 ± .0289
2-day larva	1.848 ± .0119	17.38 ± .0179	15.44 ± .0115	65.32 ± .0219
3-day larva	2.159 ± .0107	16.51 ± .0117	14.53 ± .0119	66.81 ± .0102
4-day larva	2.364 ± .0084	13.39 ± .0115	16.37 ± .0179	67.88 ± .0142
5-day larva	2.252 ± .0102	15.84 ± .0063	13.75 ± .0107	68.16 ± .0135
6-day larva	2.187 ± .0102	16.06 ± .0107	13.17 ± .0117	68.58 ± .0287
1-day pupa	2.434 ± .0177	16.50 ± .0067	10.55 ± .0332	70.52 ± .0083
2-day pupa	2.411 ± .0177	20.37 ± .0070	16.01 ± .0035	61.21 ± .0083
3-day pupa	2.346 ± .0076	19.73 ± .0089	18.91 ± .0017	59.03 ± .0107
4-day pupa	2.563 ± .0102	15.31 ± .0067	11.55 ± .0075	70.58 ± .0126
Early adult	2.257 ± .0176	15.33 ± .0019	12.25 ± .0216	70.03 ± .0107
Late adult	1.987 ± .0107	22.49 ± .0019	8.18 ± .0107	67.35 ± .0107

pupa. It then increased to 20 in the two-and three-day and then decreased to 15 per cent in the four-day pupæ and young adults. Finally it increased to 22.49 per cent in the old adults. Fraction C increased from 13.63 per cent in the one-day and to 16.37 in the four-day larvæ. This increase was followed by a decrease to 13 in the five- and six-day larvæ and to 10.55 per cent in the one-day pupa. It then increased to 18.91 per cent in the three-day pupa and decreased to 12 in the four-day pupa and young adult, and finally to 8.18 per cent in the old adult. Fraction D remained constant at about 68 per cent throughout the larval

period. It decreased to 59.03 in the three-day pupa, returning to its former level of about 70 per cent in the four-day pupa and young adult.

DISCUSSION

Many authors have reported that the process of metamorphosis in holometabolous insects is accompanied by no significant loss of nitrogen (Kellner, Sako, and Sawano 1884; and Inouye 1912, for the silkworm *Bombyx mori;* Heller 1924, for the moth *Deilephila euphorbiæ;* Frew 1929, for the blowfly *Calliphora;* Evans 1932, for the blowfly *Lucilia sericata;* Anderson 1948, for the Japanese beetle *Popillia japonica;* and others). No loss of nitrogen was evident in the present study, during the metamorphosis of *Musca domestica,* although at pupation the larval skin is shed, and upon emergence of the adult, the pupal skin is also shed. Both these exuviæ may be shown to contain nitrogen.

An examination of the data presented in Figures 2 and 3, reveals that Fraction D remains at a high value while Fraction C remains low throughout the larval period. During the pupal period Fraction D decreased and Fractions B and C increased during the second and third days. This shift in nitrogen indicates a breakdown of larval protein and an increase in the decomposition products. At the end of the pupal stage, this change is reversed indicating a utilization of these products for the synthesis of tissue. Evans (1932), for the metamorphosis of *Lucilia sericata,* also found that when the highest value for insoluble protein was obtained there was a corresponding low value of soluble protein nitrogen. In 1934, he obtained similar changes in the mealworm, *Tenebrio molitor.* Anderson (1948), found that during the metamorphosis of the Japanese beetle, pupation is accompanied by a relatively tremendous change in nitrogen distribution, water-insoluble nitrogen undergoing a marked decrease, while the water-soluble fractions show significant increases.

A possible explanation for the disappearance of Fraction C, containing the water-soluble compounds precipitated by tungstic acid, with the technique used by Ludwig and Rothstein (1952), is that when the lipid fraction is removed prior to water extraction, some of the proteins which are water-soluble and which would ordinarily be precipitated by tungstic acid are also removed. The possible removal of water-soluble protein with the

lipids would also explain the larger lipid fraction with this technique than that obtained when water extraction is made prior to lipid extraction. Furthermore, alcohol is a protein precipitating agent. Hence it precipitates some of the proteins which would also appear in Fraction C. These proteins then appear as insoluble proteins in Fraction D. A comparison of values in Table 1 with those in Tables 2 and 3 shows a greater amount of nitrogen in Fraction D with this procedure. On the other hand, when the water-soluble compounds are removed first, some of the lipid fraction may also be removed, therefore accounting for the larger percentages of these fractions, when compared with the technique of Ludwig and Rothstein (1952).

The results obtained with the three methods of fractionation indicate that little change occurs in the various fractions during the entire larval period. However, during the pupal period each method of fractionation indicated an increase in the water-soluble nitrogen during the second and third days of the pupal stage which was then followed by a steady decrease during the remainder of metamorphosis. The insoluble nitrogen decreased at the time when water-soluble nitrogen increased.

The difference in timing of metamorphosis is of utmost importance when correlating these results with the work of other investigators. Anderson (1948) reported changes which occur at or after pupation. This description suffices for the Japanese beetle, for in this insect the shedding of the last larval skin is visible. However, in the housefly, the molt which results in the formation of the pupa cannot be seen due to the presence of a puparium. Consequently, the results tabulated are timed from puparium formation and not from pupation. The latter process probably occurs within a 24 hour period after puparium formation at the temperature employed. If this 24 hour interval is subtracted from the time values given in this work for the pupal stage, the increase in water-soluble and the decrease in water-insoluble nitrogen occur at approximately the same time with respect to pupation, as that reported in the Japanese beetle by Anderson (1948).

SUMMARY

One hundred milligram samples of houseflies, *Musca domestica,* collected at 24 hour intervals during growth and metamorphosis,

at 30° C. were analyzed for nitrogen by different fractionation methods. The determinations were made of the following fractions: Fraction A (lipid nitrogen), Fraction B (water-soluble nitrogen not precipitated by tungstic acid), Fraction C (water-soluble nitrogen precipitated by tungstic acid), and Fraction D (water-insoluble nitrogen).

No changes occurred in the distribution of nitrogen among these fractions during the larval period.

No loss of nitrogen was evident in the present study. There was an increase in the percentage of total nitrogen during metamorphosis from larva to pupa due to a loss of water.

During the pupal stage, a decrease in water-insoluble nitrogen and a corresponding increase in water-soluble nitrogen occurred in the second and third days following puparium formation. The reverse shifts occurred during the latter part of the pupal stage.

These complimentary shifts between the nitrogenous fractions may be interpreted as indicative of the destruction of mature larval tissues in the early pupæ, followed by gradual construction from the materials thus made available of the definitive structures of the adult.

When lipid extraction was done prior to water extraction, some of the water-soluble proteins which would ordinarily be precipitated by tungstic acid are also removed, therefore giving a greater percentage of total nitrogen in this fraction. Also, alcohol precipitates proteins which would ordinarily be present in Fraction C, but consequently appear as insoluble proteins in Fraction D.

When water extraction was done prior to lipid extraction, some of the lipids, not tightly bound, were also removed, therefore adding to a larger value for the water-soluble fractions.

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Dr. Vishniac seconded the motion and it was carried without dissent.

Dr. Clausen appointed a nominating committee of Mrs. Vaurie, Dr. James A. Mullen and Mr. Sam Harriott.

Dr. Vishniac introduced the speaker of the evening, Dr. Louis S. Marks of Fordham University, who spoke on "Notes on the Genus Papilio". Dr. Marks traced the generic concepts within the genus from the time of Linnaeus, and presented new evidence for the creation of three genera—*Battus* Scopoli, *Papilio* Linnaeus, and *Graphium* Scopoli. He then outlined briefly our present concept of populations within the various species. His talk was illustrated by Kodachromes and an exhibit. The exhibit consisted of five boxes of *Papilio* (sensu lato). At least one specimen from each of the minor groups into which the genera are subdivided was exhibited. Noteworthy and rare swallowtails included *Papilio aristor*, *Papilio aristodemus ponceana*, the female of *Papilio alexandrae*, and a dwarfed *Papilio glaucus*.

LOUIS S. MARKS, *Secretary*

Minutes of meeting of December 8, 1953 are not available.

MEETING OF DECEMBER 15, 1953

A regular meeting of the Society was held at the American Museum of Natural History. There were nine members and six guests present.

Miss Eleanor Lappano of Fordham University was proposed for membership by Dr. Forbes.

The speakers of the evening were Dr. Roman Vishniac and Dr. Szybalski of Cold Spring Harbor Biological Labs on "Resistance in Insects and Bacteria".

Dr. Vishniac explained the phenomenon of resistance on a genetic basis. He pointed out that the resistant population has always been present, and

(*Continued on page 159*)